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## THE HARMFUL ACTION OF DISTILLED WATER<sup>1</sup>

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### HISTORICAL INTRODUCTION

The use of distilled water in experimental biological work introduced a group of problems some of which still demand the attention of the investigator. The use of this medium was begun and has continued because of the desire of the experimenter to test the effect of pure water, not only for the purpose of furnishing a check on the action of aqueous solutions of various substances under investigation, but also in order to meet the necessity of providing the pure substance for the study of the physiological action of water itself.

It was early recognized that for chemical purposes distillation furnished a convenient means of ridding water of a great part of its impurities, and for many years distilled water was accepted as being pure water, and its effects were accepted as those characteristic of pure water.

*Harmful Action Recognized.*—The essentially harmful action of distilled water was suspected, however, at a relatively early date by the plant physiologists, Sachs (1), Knop (2), Boehm (3), Dehérain (4) and others. It was the general opinion that rain water, river water and water from other natural sources exerted a more favorable action on their experimental plants than distilled water and the use of natural waters was frequently resorted to in order to secure what was regarded as normal plant behavior. This harmful action was thought to be due chiefly to the lack of nutrients and less often to actively injurious qualities.

At about the same time the deleterious effect of distilled water on

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the structure or functions of animals was noted by Kölliker (5) working on the irritability of nerves, by Nasse (6) dealing with the supporting power of various solutions for frog's muscle, and a little later by Sydney Ringer (7) working on the survival of fishes in different media.

*Harmful Impurities Sought.*—The existence of harmful properties in distilled water being recognized, many investigators working along several lines of biological and chemical study have endeavored to ascertain the reason for the results observed.

As would be expected, the search for poisonous substances in the water has been thoroughly prosecuted and many investigators have believed the problem solved by results obtained along this line.

In 1891, Loew (8) called attention to the work done on distilled water by Nägeli (9) in an investigation probably begun in the early eighties, but unpublished until 1893 after his death. In this paper, he showed that distilled water from metal stills was contaminated by compounds of the metals coming in contact with the water. These results confirmed by Loew (8), Locke (10), Ringer (11), Dehérain & Demoussy (12), Bokorny (13) and others working in diverse fields of biological research, seemed greatly to simplify the distilled water question. Water distilled from glass was regarded as a well understood and safe medium for physiological work. This comfortable conclusion, however, was not allowed to stand unchallenged.

More recently a number of investigators have asserted that water distilled from glass even with the usual precautions is harmful, through the action of impurities present. Lyon (14) and later Livingston (15) and his associates in the Bureau of Soils of the U. S. Department of Agriculture convinced themselves that distilled water thus prepared contained both volatile and non-volatile unknown poisons. Lyon suggested that dissolved ammonia might be the harmful agent.

*Harmful Properties of Pure Water.*—Early in the history of the problem it was noted by Knop (16), Zöbl (17) and others among the plant physiologists and by Paul Bert (18), Plateau (19, 20) and Sydney Ringer (7) among the animal physiologists that organisms lose salts when brought into solutions relatively lacking in these constituents and the harmful effects due to this extraction of materials was pointed out. Detailed information concerning the materials extracted was worked out by Knop (16) and Zöbl (17), while Ringer (7) hazarded some well considered surmises. This study was prolonged by Ringer (21-24) and his associates through a number of succeeding years by the use of a variety of methods and of experimental subjects.



The development of the more refined methods of physical chemistry brought forward evidence which when applied to distilled water as a beverage and as a therapeutic agent taken in by way of the stomach, aroused much discussion. Koeppe (25) maintained that distilled water was a poison when drunk in quantity and exerted its harmful action through its powerful osmotic properties. By means of these, it was contended that the cells of the stomach membranes were injured and salts were extracted from the organism with results serious to health. He was supported by Oldham (26) and others. A spirited defense by Winckler (27), Kobert (28) and others followed; the long and favorable experience of practitioners who had found distilled water a harmless and valuable remedy was pointed out. It is clear from reading the literature that while Koeppe was speaking of water considered pure from the standpoint of the physical chemist, his opponents regarded rain water, commercial distilled water and similar somewhat contaminated waters as the physiological equivalent of Koeppe's medium. The fallacy of this assumption was not realized by them.

The more recent studies of Jacques Loeb (29, 30), A. W. Peters (31) and others have been built essentially on the foundation laid by Ringer and other predecessors. It is agreed that the extraction of substances from the animals used as test objects is accomplished by distilled water, the line of argument advanced by Sidney Ringer and Koeppe being accepted in so far as the general method of working injury is concerned. The advance over their views is found to consist chiefly in the deeper penetration into the cellular operations involved. While Ringer and Koeppe were content to say that salts essential to the maintenance of the integrity of the structure and function of the organism were extracted by distilled water, Loeb seeks to relate the salts extracted to certain necessary ion-proteid compounds.

#### EXPERIMENTAL EVIDENCE

The results here given were worked out in the winter and spring of 1905, and in part in the winter of 1906. The preparation of the distilled waters and of the few solutions studied in this connection was kindly done at the writer's request by Dr. Lyman J. Briggs, at that time soil physicist of the Bureau of Soils, U. S. Department of Agriculture. The help of Dr. G. F. Klugh, at that time the writer's assistant, was obtained during the winter of 1906. The results and



conclusions here given are regarded as a preliminary contribution to the solution of the problem.

*Materials and Methods.*—The distilled waters were prepared in Dr. Briggs's laboratory, under his supervision. As test objects, the radicles of *Lupinus albus*, the white lupine, were chosen. This genus had been shown by Frank (32) to be very sensitive to distilled water injury, a fact rendering it an especially favorable subject for study in this connection. The radicles were suspended in beakers containing the culture solutions, glass hooks being used so to hold the seedlings as to suspend them with their roots in the solutions. These hooks were supported by a cork sheet covering the whole beaker through which they were thrust. The beakers used were made of glass of the so-called "nonsol" formula and had a capacity of 300 c.c. About 150 c.c. of the solutions were used in each culture of four roots. These beakers had never been used, and were prepared by being thoroughly washed and steamed out. In order to get numerical data for comparison, the growth rate of the roots was adopted as a criterion, and the standard period of time between measurements was twenty-four hours. A fine line of India ink was fixed at a distance of 15 mm. from the tip, and the change in length of this region included the entire growth in length of the radicle. The seeds were germinated in moist, chopped sphagnum carefully washed out, and the seedlings before setting into the culture medium were carefully rinsed in a duplicate portion of the same.

As a check medium, tap water was chosen. This is drawn from the Potomac River, some miles above the city, filtered on a large scale, and delivered to the city through the usual appliances for distribution. Analyses of filtered Potomac water covering the entire period of our experiments are not available, but an average of the analyses made by Outwater (33) covering twelve months, between May, 1904, and August, 1905, is as follows:

	Parts per million
SiO <sub>2</sub> .....	5.15
F <sub>2</sub> O <sub>3</sub> , Al <sub>2</sub> O <sub>3</sub> .....	4.63
Ca.....	30.94
Mg.....	4.62
Na.....	3.20
K.....	0.62
Cl.....	5.02
SO <sub>4</sub> .....	8.68
CO <sub>3</sub> .....	2.03
HCO <sub>3</sub> .....	97.77



During this period, the Ca content varied between 10.37 parts (May, 1904) and 54.41 parts per million (Dec., 1904); the Mg content between 1.87 parts (May, 1904) and 8.29 parts (Dec., 1904); the K content between a "trace" during 9 months of the period and 5 parts per million in June, 1904.

The beakers containing the cultures, when not under observation, were shut in a dark laboratory cupboard, where the temperature was maintained between 20° and 22.5° C.

Owing to the limits set on the development of lupine roots by distilled water, extended experiments could not be carried out. It is clear, therefore, that the behavior of plants during long periods should be investigated with peas, maize, or other species which are less sensitive to the influences exerted by distilled water.

#### EFFECT OF USING CONDENSERS MADE OF DIFFERENT MATERIALS

It was thought desirable, first of all, to establish the relation between the growth rate in Potomac water and in distilled waters made by using condensers of various materials. Accordingly, water was distilled from glass, the vapors being condensed in copper, tin, platinum and glass condensers, respectively. The resulting waters were tested in two different experiments, the following summary presenting the average of the two series of plants:

TABLE I  
WATERS CONDENSED IN DIFFERENT MATERIALS  
Temp. 20° to 22.5° C.

Source of water	Growth 1st 24 hrs., mm.	Growth 2d 24 hrs., mm.
Copper Condenser.....	14.5	6.2
Tin Condenser.....	15.7	6.5
Platinum Condenser.....	18.2	6.7
Glass.....	16.2	6.5
Potomac water.....	23.5	27.5

From this experiment it is clear that all distilled waters were distinctly harmful in their action during the first 24-hour period, the advantage being with the platinum condenser. According to Nägeli (9), Loew (8) and others, glass apparatus is supposed to deliver physiologically safe distilled water. It is of interest in this connection to note that Copeland and Kahlenberg (34) found that lupine roots



grown in containers in which considerable areas of pure platinum metal were allowed to remain throughout the duration of the experiment showed a greater growth after 11 days than did the roots in the control culture of distilled water. Copper is here the most harmful. During the second 24-hour period, no clear advantage seems to lie with any kind of material. The cause of damage present in all of these distilled waters continues to operate throughout the period of the experiment.

In view of the harmful action of the water distilled from glass, it seems to follow that metal carried over from the apparatus can hardly be invoked as the fundamental source of trouble.

#### EFFECT OF DISTILLATION PROCESS COMPARED WITH FREEZING OUT

It being clear that all waters purified by distillation were harmful compared with tap water, the question next arose as to whether the process of distillation itself, in the various kinds of apparatus used, was the source of trouble, or whether water purified by other means would act like distilled water. Accordingly, clear, natural ice was melted and refrozen three times in vessels containing no metal, the water not being allowed to warm to room temperature until after the last recrystallization. Comparison cultures of distilled water condensed in copper and platinum and a check in Potomac water were set up.

TABLE II

##### WATERS PURIFIED BY DISTILLATION AND BY RECRYSTALLIZATION

Method of purification	Growth 1st 24 hrs., mm.	Growth 2d 24 hrs., mm.
Recrystallization.....	13.0	5.0
Copper condensed.....	15.0	7.5
Platinum condensed.....	15.0	6.4
Potomac water.....	20.7	17.0

It appears from these results that water purified by repeated recrystallization is fully as harmful to lupine roots as that purified by distillation. This result suggests two explanations of the cause of trouble—either the water is harmful because of traces of toxic substances left in it by both the recrystallization and the distillation processes, or it is more harmful than in its original state because of the loss of substances there present.



*Leaching Action of Distilled Water.*—It has long been known that seeds, when soaked for long periods in water, give up salts to the water in quantities sufficient to permit analysis by the ordinary methods. This work has been repeated and extended by André (35, 36) to include potato tubers as well as seeds, and it seems clear that all of these structures give off salts to relatively pure water. Sachs (37) at an early date showed that this conclusion should be extended to include leaves also.

Physiologists working on animals have made it appear probable that distilled water is able to withdraw salts from animal tissues also, and have advanced the view that harmful results seen to follow a prolonged stay in distilled water are due to the leaching of salts required for the maintenance of normal conditions in the tissues.

The responsibility of distilled water for certain untoward catarrhal conditions of the stomach supposed by some to be associated with the prolonged use of distilled water has been much debated. It is stated by Findlay (38) and others that when copiously used, distilled water leaches the salts and perhaps other materials from the stomach linings with resulting injury to the membranes. On the other hand, distilled water has found active defendants who contend that it is harmless when thus used.

Since it seemed doubtful in the course of the above investigations that the harmful results produced by distilled water on lupine seedlings could be laid at the door of impurities dissolved in the water, attention was given to the alternative possibility that the water was harmful because of its lack of dissolved substances. Again, the electrical conductivity of the water was used as a means of ascertaining the degree of purity. Of course, it need hardly be pointed out that this test is at best but an imperfect one, since non-electrolytes might be present in the solution and remain undetected. Indeed Knop (2) has shown that distilled water extracts some organic as well as inorganic constituents from seeds of peas and corn. Since, however, there was no better method available, it was accepted as affording valuable data.

Accordingly, a series of cultures was set up which were designed to test the supposition that salts might be leached from the roots. It seemed likely that if leaching takes place, a greater amount of salts would be withdrawn into an outside volume of 150 c.c. of distilled water from several roots than from a less number.



TABLE V  
LEACHING ACTION OF DISTILLED WATER IN 24 HOURS

Medium	Number of roots	Growth in 24 hrs., mm.	Electrical conductivity $\times 10^{-4}$
Distilled water.....	10	....	2.32
Distilled water.....	4	11.2	1.43
Distilled water.....	0	....	0.82

This result shows that a volume of distilled water to which four lupine roots are exposed for 24 hours gains markedly in conductivity in comparison with a like volume of the same medium standing under like conditions, but containing no roots. The increase in conductivity per root is about  $0.151 \times 10^{-4}$  in twenty-four hours. When instead of four roots ten are present in the medium, the total fall in resistance is distinctly greater, indicating the corresponding leaching action of the distilled water. It is to be noted also that the rate of increase in conductivity per root is about the same as that seen in the series of four individuals, viz., about  $0.155 \times 10^{-4}$ .

It was next desired to test this same action over longer periods of time, in order to ascertain whether the extraction process was continued.

Cultures containing 150 c.c. were set up, containing ten roots, four roots, and no roots, in three series, to run for 48 hours and 72 hours, respectively.

TABLE VI  
LEACHING ACTION OF DISTILLED WATER DURING 48- AND 72-HOUR PERIODS

Medium	Duration of test, hrs.	No. of roots in culture	Growth rate			Electrical conductivity at end of period $\times 10^{-4}$
			1st 24 hrs., mm.	2d 24 hrs., mm.	3d 24 hrs., mm.	
Distilled water....	48	10	....	....	....	4.35
	72	10	....	....	....	4.76
Distilled water....	48	4	9.5	2.5	....	1.87
	72	4	9.5	3.0	2.5	2.06
Distilled water....	48	0	....	....	....	0.80
	72	0	....	....	....	0.75

This table shows that both in the cultures containing 4 roots and 10 roots, respectively, the leaching of ions into distilled water continues, but at a slightly diminished rate in comparison with that seen in the first 24-hour period, the total leaching for the 48-hour period being relatively greater than for the 72-hour period.



In order to bring out the relative rate of extraction more clearly, the following table is compiled from the foregoing data. It shows the rate of leaching as gain in conductivity expressed in units per root per day of 24 hours.

TABLE VII  
RATES FOR LEACHING PER ROOT PER DAY

Time of experiment	Four roots	o ts
24 hours.....	$0.151 \times 10^{-4}$	$0.155 \times 10^{-4}$
48 hours.....	$0.135 \times 10^{-4}$	$0.177 \times 10^{-4}$
72 hours.....	$0.110 \times 10^{-4}$	$0.134 \times 10^{-4}$

This comparison shows that in the experiment with 4 roots the leaching continues through the longest period tested, 72 hours, but at a clearly decreasing rate. The regularity seen here is not found in the experiment with 10 roots. It is interesting to note in this connection that the conductivity of the check decreases during the longer periods, a point to which attention will be recalled later. It is important in this connection to note that the growth rate of the culture containing 4 roots falls off in a manner suggesting a parallel with the decrease in resistance. As the distilled water progressively extracts electrolytes, and perhaps other substances, from the plant the growth rate diminishes and almost ceases. In view of the fact demonstrated in earlier studies that the primary radicle of the white lupine seems to be unable to recover in distilled water, seedlings were not exposed for a longer period.

The results seen above seem to indicate that the leaching process begins actively when the roots enter the solution, and during the 72-hour period in which the roots remain there continues at a somewhat decreasing rate. It was thought desirable to follow somewhat further the course of the changes in conductivity and to try to ascertain whether with the accumulation of the leached materials the extraction of the plants continued. Accordingly cultures were arranged in which six series of plants were used: (1) In 150 c.c. of distilled water, 4 plants; (2) in the same volume, 10 plants; (3) in the like volume, no plants; (4) in the like volume of Potomac water, 4 plants; (5) in the same volume of Potomac water, 10 plants, and (6) in the same volume of Potomac water, no plants. Since it was desired to test the physiological properties of these media as affected by the gain of solutes from the leached roots or by the loss of dissolved material through



the absorption of such by the roots, each culture container received a new set of seedlings at the end of each three days. This period was chosen since, judging by the growth rate and appearance, it was deemed as long a period as lupine roots could be relied upon to survive the action of distilled water. Post mortem leaching was expressly avoided since it would greatly complicate the situation. Conductivity readings were made at the end of each 72-hour interval. In the case of the check cultures of distilled water and of Potomac water in which no plants were placed, the first readings given represent the conductivity of these latter waters at the beginning of the experiment.

The general features of the result stand out clearly. As the distilled water continues to receive the successive lots of roots the leaching of these structures continues throughout the experiment, as is shown by the steadily increasing electrical conductivity.

The conductivity of the culture containing 10 roots rises more rapidly than that containing 4 roots. At the close of the experimental period of 21 days the conductivities representing the total result of the changes of each culture were as follows: The check containing no roots had risen from a conductivity of  $0.87 \times 10^{-4}$  to  $1.61 \times 10^{-4}$ , or in terms of the equivalent concentrations of KCl from  $0.87M/45,500$  to  $1.61M/45,500$ , an increase of  $0.74M/45,500$  KCl. The culture containing 4 roots showed an increase in conductivity from  $0.87 \times 10^{-4}$  seen in the water before receiving the plants to  $7.4 \times 10^{-4}$  corresponding to a change from  $0.87M/45,500$  to  $7.4M/45,500$ , an increase of  $6.53M/45,500$  KCl. Since during this interval the check attained a conductivity of  $1.61M/45,500$  from sources apart from the plants, the roots had contributed the equivalent of  $5.8M/45,500$  KCl to the solution, or about  $1.45M/45,500$  KCl per root. The culture containing 10 roots showed a change in conductivity from  $0.87M/45,500$  to  $12.9M/45,500$ , a total gain due to the roots of  $11.3M/45,500$  KCl, or about  $1.13M/45,500$  per root. It will be noted that the leaching process seems to have been somewhat less active in the culture containing the larger number of roots than in that containing the smaller number.

The plants in the Potomac water check series absorbed electrolytes throughout the entire period of the experiment, and before the close of the interval of observation the river water culture containing 10 roots contained a less quantity of electrolytes than either of the distilled water cultures at the same time and the Potomac culture containing 4 roots contained approximately the same quantity of electro-



TABLE VIII  
LEACHING ACTION OF DISTILLED WATER DURING LONGER PERIODS

Medium	Number of plants used	Total growth 72 hours, mm.	Electrical conductivity $\times 10^{-4}$
Dist. water.....	4 (1st set)	13.0	2.08
Dist. water.....	10 (1st set)	12.0	3.85
Dist. water.....	0	....	0.87 <sup>2</sup>
Potomac water.....	4 (1st set)	25.0	30.3
Potomac water.....	10 (1st set)	26.0	24.1
Potomac water.....	0	....	37.0 <sup>2</sup>
Dist. water.....	4 (2d set)	9.0	2.94
Dist. water.....	10 (2d set)	11.5	6.94
Dist. water.....	0	....	0.86
Potomac water.....	4 (2d set)	26.0	23.8
Potomac water.....	10 (2d set)	21.5	15.9
Potomac water.....	0	....	33.3
KCl. <i>M</i> /1,000.....	.....	....	40.0 ?
Dist. water.....	4 (3d set)	9.5	3.85
Dist. water.....	10 (3d set)	12.0	8.00
Dist. water.....	0	....	0.97
Potomac water.....	4 (3d set)	23.0	22.2
Potomac water.....	10 (3d set)	21.0	12.2
Potomac water.....	0	....	34.5
KCl. <i>M</i> /1,000.....	.....	....	45.0
Dist. water.....	4 (4th set)	10.5	4.76
Dist. water.....	10 (4th set)	12.5	9.05
Dist. water.....	0	....	1.12
Potomac water.....	4 (4th set)	25.5	19.6
Potomac water.....	10 (4th set)	20.5	8.7
Potomac water.....	0	....	35.7
KCl. <i>M</i> /1,000.....	.....	....	45.4
Dist. water.....	4 (5th set)	11.7	5.46
Dist. water.....	10 (5th set)	13.5	9.80
Dist. water.....	0	....	1.22
Potomac water.....	4 (5th set)	26.5	17.5
Potomac water.....	10 (5th set)	24.4	9.5
Potomac water.....	0	....	37.0
KCl. <i>M</i> /1,000.....	.....	....	45.6
Dist. water.....	4 (6th set)	10.0	6.41
Dist. water.....	10 (6th set)	11.0	11.4
Dist. water.....	0	....	1.37
Potomac water.....	4 (6th set)	21.7	15.0
Potomac water.....	10 (6th set)	17.7	4.31
Potomac water.....	0	....	37.0
KCl. <i>M</i> /1,000.....	.....	....	45.6
Dist. water.....	4 (7th set)	18.5	7.4
Dist. water.....	10 (7th set)	17.0	12.9
Dist. water.....	0	....	1.61
Potomac water.....	4 (7th set)	46.0	13.5
Potomac water.....	10 (7th set)	27.0	4.65
Potomac water.....	0	....	38.4
KCl. <i>M</i> /1,000.....	.....	....	45.4

<sup>2</sup> Determinations made at the beginning of the experiment, representing original conductivity of these waters.



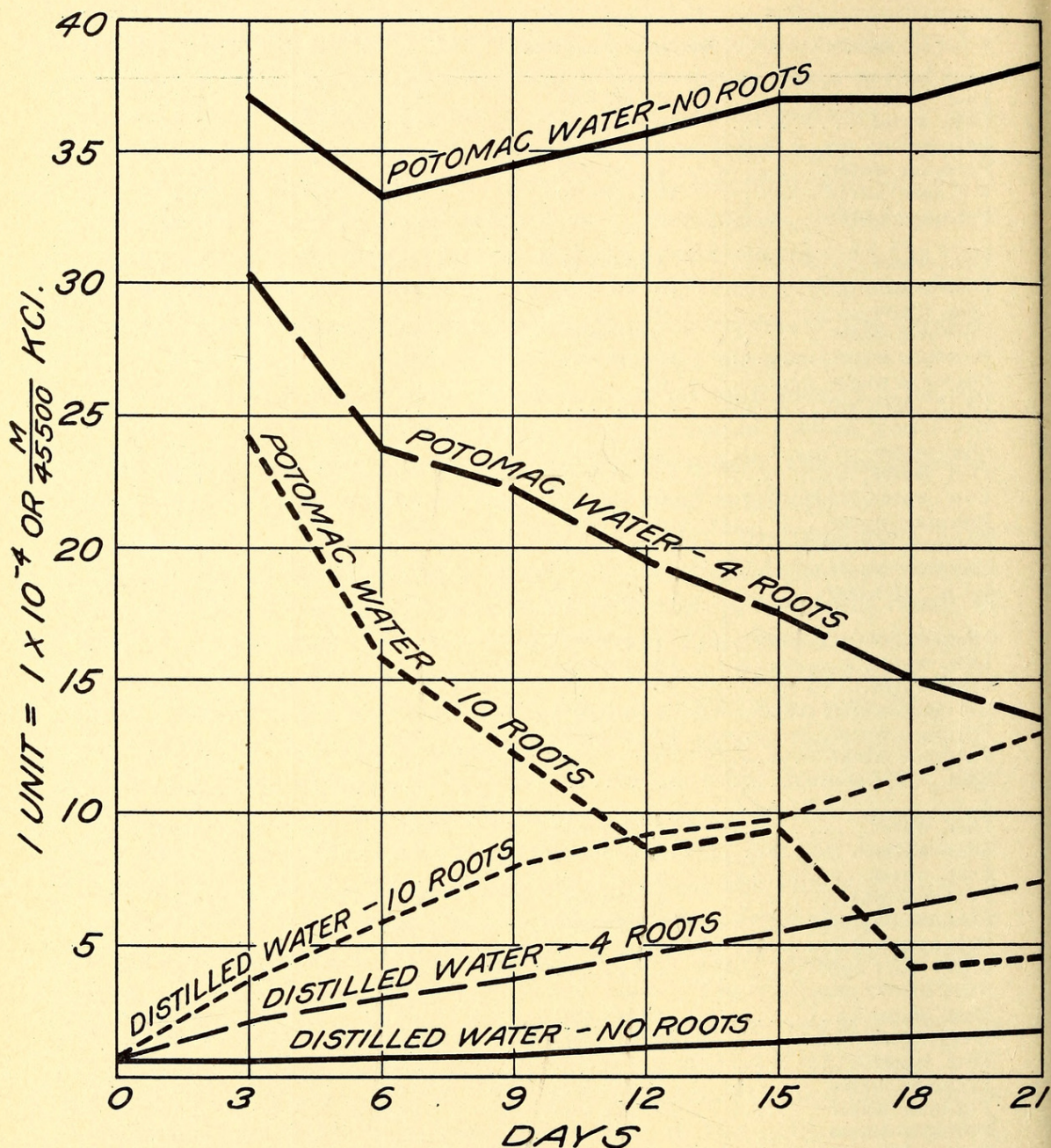


FIG. 1. Changes in the conductivity of distilled water and of Potomac water due to their action on lupine roots.

lytes as the distilled water culture containing 10 roots, and only about twice the quantity found in the distilled water containing 4 roots.

The distilled water check containing no roots showed a slight though steady rise, due largely perhaps to the gradual solution of the



glass container. The Potomac water check showed more irregularity in its behavior due to causes not determined, the result being a gradual rise in conductivity following a rather abrupt fall early in the experiment. This first abrupt fall is possibly due to the breaking down of  $\text{CaN}_2(\text{CO}_3)_2$  with the formation of insoluble  $\text{CaCO}_3$ .

In order to make the general features of the experiment clearer, the accompanying curves have been platted. On the axis of ordinates one unit equals a conductivity of  $1 \times 10^{-4}$  corresponding to a solution containing  $M/45,500$  KCl. On the axis of abscissas five space units equal an interval of three days. (Fig. 1.)

It has now been shown that water of a rather high degree of purity is harmful to the roots of lupine seedlings suspended in it; that the growth rate of these roots rapidly falls off and at the end of a period of three days is almost zero; that at the same time the roots continually give up electrolytes to the water, and the causal connection between the loss of electrolytes by the roots and their falling growth rate is regarded as almost certain. The check cultures carried on in Potomac water containing small amounts of the common salts present in the soil show a probably normal growth rate accompanied by an absorption of electrolytes from the river water.

The question was next raised whether with the addition of soluble substances in quantities sufficient to make the water cultures osmotically equivalent to Potomac water, the growth rate of the plants would be favorably affected. Accordingly, cultures of cane sugar, calcium chloride and sodium chloride were set up having an osmotic value equal to that of Potomac water.

TABLE IX  
ACTION OF ISOTONIC SIMPLE SOLUTIONS

Description of medium	Growth rate		Electrical conductivity at end of 48 hrs.
	First 24 hrs., mm.	Second 24 hrs., mm.	
Distilled water + cane sugar. . . . .	13.0	4.5	$1.39 \times 10^{-4}$
Distilled water + sodium chloride. . . . .	19.0	5.5	$20.8 \times 10^{-4}$
Distilled water + calcium chloride. . . . .	27.0	22.0	$21.7 \times 10^{-4}$
Distilled water. . . . .	12.5	5.0	$1.22 \times 10^{-4}$

This experiment seems to show that the harmful action of distilled water as reflected in the growth rate is not due to the aggregate difference in osmotic pressure between the cells of the roots and the ex-



ternal medium, since the sugar introduced equals the osmotic aggregate of the river water. This substitution seems to leave the water with harmful properties undiminished, although sugar in itself at this concentration can hardly be called distinctly harmful. As was observed by Loeb (30) in his work on *Gammarus*, one finds here no marked improvement following the addition of the sugar. Indeed, the main features of the experiment strikingly resemble those of the check culture in which distilled water alone was used. Leaching was not hindered by the presence of the sugar but was perhaps somewhat accelerated.

The addition of sodium chloride in quantity osmotically equal to the dissolved material in Potomac water exerts a marked beneficial action in the first 24 hours, but this seems to be lost during the second day. It is obvious that although the presence of this salt protects the roots to a certain degree, the protection is of a decidedly temporary nature. It also confirms the conclusion based on the action of the sugar solution that simple aggregate osmotic pressure is not the most important feature in the case.

The addition of calcium chloride in osmotically equal quantity produces a strikingly different result. The growth rate assumes an apparently normal character throughout the course of the experiment, the peculiar "distilled water" action being absent. Since the Potomac water approaches in conductivity to a  $M/1,000$  KCl solution, giving a conductivity under the conditions of these experiments of about  $37 \times 10^{-4}$  reciprocal ohms as against  $44.5 \times 10^{-4}$  like units for  $M/1,000$  KCl, it appears that the sugar solution behaves essentially like distilled water in showing an increasing conductivity on account of the extraction of electrolytes from roots. This assumes that the sugar is not significantly conductive under these conditions. It is also clear from the higher resistances that roots absorb electrolytes from the isotonic solutions of NaCl and  $\text{CaCl}_2$  in a way comparable with the absorption from Potomac water in the foregoing experiments (p. 266). In the case of these isotonic salt solutions, it is probable in the absence of considerable quantities of other electrolytes in the distilled water, that the absorption of NaCl and  $\text{CaCl}_2$  takes place. If such is the fact, the growth rate during the second day should reflect to a certain degree the effect of such absorption. If this assumption is justified, the absorption of NaCl is less favorable than the absorption of  $\text{CaCl}_2$ . On the other hand this difference in effect may be due to



the different effects these salts have on the leaching of the cells. There seems to be good reason for thinking that abundant leaching of certain classes of compounds present in the cell would take place in the presence of but a single salt in the exterior medium.

The experiment above described was repeated with a number of variations.

TABLE X  
ACTION OF ISOTONIC SIMPLE SOLUTIONS

Description of medium	Growth rate		Electrical conductivity at the end of 48 hrs.
	1st 24 hrs., mm.	2d 24 hrs., mm.	
Distilled water + cane sugar.....	13.0	5.0	$1.0 \times 10^{-4}$
Distilled water + NaCl.....	17.0	9.0	$18.9 \times 10^{-4}$
Distilled water + $\text{CaCl}_2$ .....	24.0	12.0	$18.9 \times 10^{-4}$
Distilled water + $\text{Ca}(\text{NO}_3)_2$ .....	22.0	15.0	$18.9 \times 10^{-4}$
Distilled water + $\frac{1}{2}$ equiv. NaCl $\frac{1}{2}$ equiv. $\text{Ca}(\text{NO}_3)_2$ .....	23.0	16.0	$18.9 \times 10^{-4}$
Distilled water.....	15.0	8.0	$1.25 \times 10^{-4}$
Potomac water.....	24.0	22.5	$15.4 \times 10^{-4}$

Again the inefficiency of cane sugar as a means of improving physiological conditions is marked. The half-way benefit of NaCl again appears and the great help due to the presence of Ca salts is apparent. That it is due to the action of the Ca ion is clear from the fact that the benefit is derived from both Ca salts in spite of the fact that different anions accompany Ca. The falling off of the growth rate in the Ca-containing solutions during the second day may indicate among other possibilities that the Ca solutions fail to supply necessary needs for more than a short time or that they may fail to check the undetected leaching of small quantities of necessary substances. When one half of the osmotic equivalent of Potomac water is supplied by NaCl and one half by  $\text{Ca}(\text{NO}_3)_2$ , the record made by the roots is essentially like that produced by the Ca salt alone in full equivalent. This may mean that the Ca action is so marked even at this very considerable dilution (nearly equivalent electrolytically to  $M/2,000$  KCl solution) as to cover up the less favorable action of the NaCl present and give it in effect a Ca effect.

The check cultures in distilled water and in Potomac water serve to illustrate the great difference for *Lupinus albus* between the distilled water and Potomac water as culture media. They show clearly that



distilled water should be used with caution in laboratory work as a check medium intended to present a norm of plant action. Potomac water certainly furnished more favorable and natural conditions for root growth in the plant here concerned than distilled water. It should be noted, however, in this connection that all plants are not equally sensitive to injury from this source, it having been shown by True and Bartlett (39) that Canada field peas make a fairly healthy growth in distilled water in spite of the fact that they lose a considerable quantity of electrolytes to the outer medium.

#### SUMMARY OF RESULTS

It appears probable from the results given in the course of this paper that the problem of injury by distilled water is not a simple one capable in all cases of a like explanation. In some cases, distilled water obtained from apparatus having copper surfaces exposed to contact with the water undoubtedly derives certain toxic properties from minute traces of copper. In other cases doubtless it is possible for other harmful impurities to find their way into the product, but after the action of all the impurities has been accounted for there still remains a residuum of harmful action due to no known type of impurity. This mode of harmful action seems to be most marked in water which shows the highest resistance to the passage of the electric current.

It is shown here that those samples of distilled water which show the highest resistance are in general more harmful to lupine roots than waters containing a large quantity of electrolytes. It is likewise shown that these same samples of water withdraw electrolytes from the tissues of the roots when they remain in the water. This leaching of electrolytes is shown to be the probable mechanism by means of which purer samples of distilled water exert their harmful action on the roots. This extraction by distilled water is regarded as but a special case of the general type of injury wrought on cells by unbalanced solutions whereby certain necessary constituents, undoubtedly in part inorganic, are dissociated from their proper attachments in the complicated chemical and physical mechanism of the living cell. The distilled water seems to withdraw material required for the maintenance of the efficient action of the protoplasmic limiting membranes with the result that the permeability of the cells is increased, and a further dissociation of electrolytes from their points of combination



in the proteids, and other chemical structures of the cell ensues. These dissociated electrolytes escape from the cell and increase the conductivity of the distilled water. When a calcium salt is added to the distilled water sufficient to make it osmotically equal to tap water, this dissociating power of the distilled water over the proteids and other chemical mechanisms of the cells is largely undeveloped, and the chemical integrity of the cells is protected in some way not known.

This report is preliminary in its nature and is to be followed at a future date by a further contribution reporting the results of work now under way.

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